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An Inexpensive Alternative Bath System for Electrophysiological Characterization of Isolated Cardiac Tissue

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Abstract—**A tissue bath system, to be used as an alternative to complex perfusion chambers, was constructed for use in cardiac electrophysiological studies. This system consists of an acrylic chamber to hold circulating physiological medium such as DMEM, suspended in a water bath warmed by a hot plate. Temperature and pH were controlled to mimic physiological conditions. Rat and porcine cardiac tissues, were used to test viability of the conditions presented in the bath system. Using a cardiac mapping system, the tissues were stimulated and responses recorded. From the recordings we were able to calculate conduction velocities and spatial dispersion of activation indices. The results are comparable to previous in-vivo work, which suggests that the tissue bath system design can maintain tissue viability. This tissue bath system is a relatively simple alternative for ex-vivo testing of cardiac tissues.**

Index Terms—**Isolated tissue, ex-vivo characterization, cardiac mapping.**

I. Introduction

A myocardial infarction (MI), more commonly referred as a "heart attack", is a condition that occurs due to a period of disrupted blood supply to a portion of the heart resulting in tissue death. Every year in the United States, there are an estimated 610,000 new and 325,000 recurrent myocardial infarctions [1]. Patients suffering from MI are at risk for mechanical and electrical complications such as cardiogenic shock due to insufficient perfusion of oxygen and nutrients to the tissue. This can lead to arrhythmias or rupture of the interventricular wall, both common conditions following MI. Recently, scientists have shown interest in the research of tissue engineered cardiac patches as an alternative to treat myocardial infarction and restore normal cardiac function [2], [3]. The development of tools and techniques capable of acquiring electrophysiological and pharmacological properties and functionality of isolated ex-vivo cardiac tissues for comparative analysis is becoming more common.

A standard way to characterize these ex-vivo tissues is through the use of perfusion chambers for isolated tissues [4], or the use of the Langendorff perfused model for small animal hearts, often utilized in combination with an electrode array [5], an optical mapping system with voltage sensitive dye [6], or one lead ECG recordings [7]. These methods, however, are usually expensive and require specialized instrument systems.

The objective of this project was to design and test an inexpensive, simple alternative for a tissue bath system that can be used to characterize the electrical properties of isolated ex-vivo heart tissues in physiologically relevant conditions.

II. Methods and Materials

A. Construction of tissue chamber

The bath system was constructed to include a rectangular acrylic chamber equipped with tissue clamps and supports on each corner. The chamber was filled with enough Dulbecco's Modified Eagle Medium (DMEM, Fisher Scientific) to cover the tissue sample by 1-2 mm and was continuously circulated at 120 mm/min using a Masterflex tubing pump. Temperature inside the chamber was maintained at 37±0.5 ºC using a ceramic hotplate (C-MAG HP7 IKA®) and a water bath (metal pan filled with water). The acrylic chamber was set inside the metal pan with the water level high enough to surpass the medium level inside

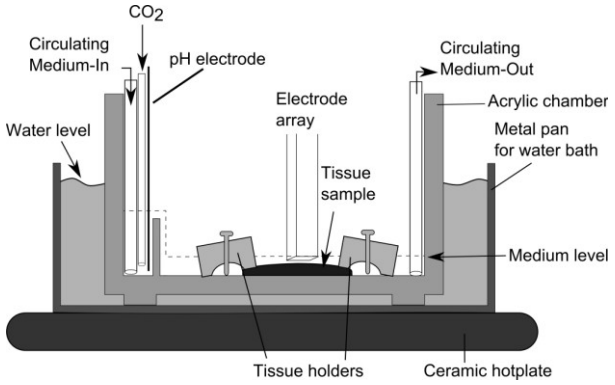

Fig. 1. Cross sectional view of the constructed bath system for electrophysiological characterization.

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the tissue chamber. The pH was maintained at 7.32±0.05 by bubbling in CO_2 using a pH controller (BL931700, Harvard Apparatus) (Fig. 1).

B. Evaluation of cardiac tissue

In order to evaluate the capability of maintaining relevant physiological conditions with our system, we tested rat and porcine cardiac tissues. A rapid heart extraction after isoflurane anesthesia was performed via median sternotomy on four male Sprague Dawley rats weighing between 354 and 413 g. Porcine samples were obtained from four cross bred pre-natal piglets weighing between 500 and 1200 g, 93% of term (115 days); piglets were euthanized using an injection of Butler Somnasol Euthanasia-III solution 9-10 days after birth. Both sets of excised hearts were then rinsed in warmed 37 ºC saline solution, dissected through the right ventricular wall to allow the tissue to lay flat with the epicardium of the left ventricle facing up, and subsequently submerged inside the tissue bath system.

Tissue was constantly assessed visually for viability. This judgment was based on color, texture, coagulations and hardness of the tissue; the experiment was terminated when tissue hardened and no further electrical response was observed after stimulus.

Each tissue sample was stimulated and electrograms were recorded using our current cardiac mapping system [8], consisting of an array of 14x14 Ag/AgCl coated copper electrodes connected to a data acquisition card with LabVIEW interface capable of recording independent analog signals in each interior electrode (192 total). The pacing threshold was determined using a 3 ms pulse width square wave stimulus at 1 Hz for five second duration, delivered from a line of electrodes bordering one side of the electrode array situated at the base of the heart. The initial stimulus amplitude was 0.5 mA, increasing by 0.1 mA until an active response was achieved. The tissues were stimulated with pacing episodes of 5, 10, 20, 60, and 300 seconds at 1.5 times the pacing threshold value; temperature and pH were recorded at the end of each pacing episode.

C. Data Analysis

The activation time for each electrode was determined as the minimum value of the first derivative from each electrogram recorded during pacing [9]. Pacing episodes were then analyzed for electrical wavefronts and activation contour maps were obtained. Conduction velocity vector fields and mean conduction velocities (CV) for each episode were estimated using MATLAB as in [10]. Spatial dispersion of activation index (DI) was calculated in terms of the 5th, 50th and 95th percentiles (P_5, P_{50}, P_{95}, respectively) of the maximum difference between activation times according to (1) [11].

$$DI = \frac{P_{95} - P_5}{P_{50}}. \qquad (1)$$

TABLE I
SUMMARY OF EXPERIMENTS AND COMPUTATIONS

	Rat samples	Porcine samples
Number of animals	4	4
Successful pacing episodes (N)	25	25
Mean CV (m/s)	1.14±0.21	0.79±0.24
Mean DI (ms)	1.06±0.24	1.47±0.34

III. RESULTS

Temperature and pH were maintained in the appropriate range with little fluctuation (37±0.5 ºC and 7.32±0.05 respectively). Both samples visually maintained a healthy color and responded to pacing stimulus throughout the experiment protocol, approximately 40-50 minutes.

An active response was successfully achieved in the samples and data analysis is summarized in Table 1.

The activation wavefronts were consistent in the pacing episodes. Figure 2 shows the activation time contour map for one pacing episode from two animals along with the respective conduction velocity vector field.

IV. DISCUSSION

The results obtained for mean conduction velocity and DI are comparable to previous studies [12-15].

The use of tissue baths is not as common as the use of the Langendorff model for ex-vivo electrophysiological studies, but still proves to be an accessible and effective method for those researchers interested in cardiac mapping. Bath systems, like the one designed in this study, present an alternative that could also be used for tissue-engineered cardiac patches. It is still crucial to control temperature and pH, since these parameters must be in balance to produce consistent measures of electrophysiologic characteristics [16].

V. CONCLUSIONS

Our system provides an accessible and reliable option for mapping of different cardiac tissues. A majority of the elements used are readily available to most labs, therefore the acquiring of new equipment may be only limited to obtaining a pH control method. The use of tissue engineered characterization techniques will play an important role in the near future, as many researchers are developing therapies requiring cultured cells, scaffolds, and patches designed for implantation in the human body and the interest in ex-vivo electrophysiology and pharmacology studies continues increasing.

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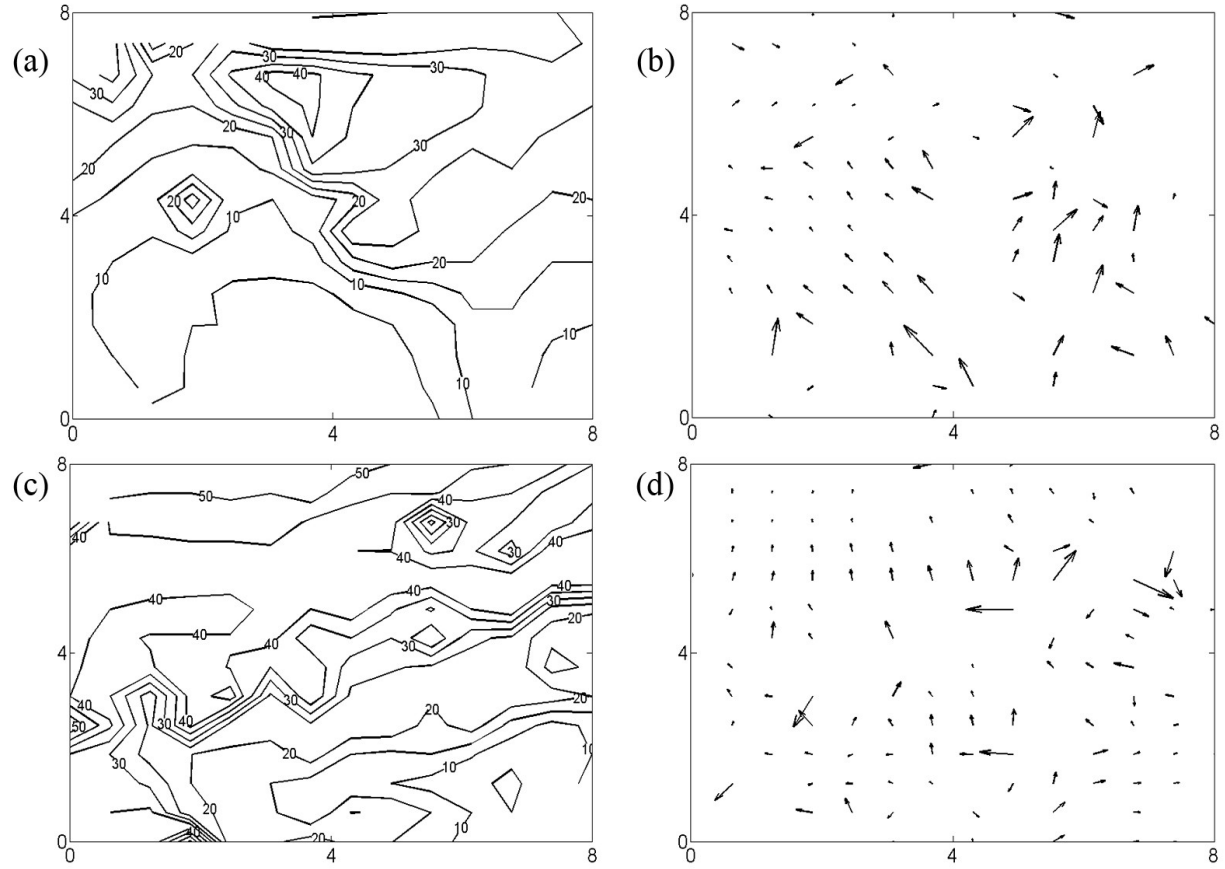

Fig 2. Activation time contour maps after pacing (ms) and conduction velocity vector fields for rat (a) and (b), and porcine (c) and (d) samples, respectively. Conduction velocity vector length is proportional to conduction velocity magnitude. Stimulation was delivered from the bottom edge of each map which corresponds to the base of the heart. The 14x14 electrode array is 8x8 mm.

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